In Vitro Bile-Acid-Binding of Whole vs. Pearled Wheat Grain

T. S. Kahlon,[1,2] M. M. Chiu,[1] and M. H. Chapman[1]

ABSTRACT

Health benefits of consuming whole grains are reduced risk of heart disease, stroke, and cancer. The U.S. Health and Human Services and USDA dietary guidelines recommend consumption of 6–10 oz of grain products daily and one-half of that amount should contain whole grains. Whole grains contain vitamins, minerals, fiber, and phytonutrients. Bile-acid-binding capacity has been related to cholesterol lowering potential of food fractions. Lowered recirculating bile acids results in utilization of cholesterol to synthesize bile acid and reduced fat absorption. Secondary bile acids have been associated with increased risk of cancer. Bile-acid-binding potential has been related to lowering the risk of heart disease and that of cancer. It has been reported that bile-acid-binding of wheat bran is not related to its total dietary fiber (TDF) content. Whole (W) grain as well as pearled (P) hard red winter wheat (Hrw), hard white winter wheat (Hww), and durum wheat (DU) cooked grains were evalu-ated for in vitro, bile-acid-binding relative to cholestyramine (a choles-terol lowering bile-acid-binding drug). On dry matter basis (db) relative bile-acid-binding values were 7.7% WHrw; 7.5% WHww; 6.3% PHww; 6.0% PHrw; 5.5% WDU; and 5.4% PDU. On a TDF basis, binding values were 42–57% of that for cholestyramine for the whole and pearled wheat grains tested. Bile-acid-binding values (db) for WHrw and WHww were similar and significantly higher than those of PHww, PHrw, WDU and PDU. Similar bile-acid-binding of WHww to that of WHrw suggest that the red color commonly associated with whole grain may not necessarily indicate more healthful potential. Data suggest that cooked WHrw and WHww wheat have significantly higher health-promoting potential than pearled grains. WDU or PDU wheat health-promoting potential was simi-lar to that of PHww or PHrw. Consumption of products containing WHrw and WHww are recommended.

The 2005 Dietary Guidelines and new Food Guide Pyramid (http://www.mypyramid.gov) recommend eating 6–10 oz of grain products daily and one-half of that should contain whole grain (NHANES 2005). This recommendation is based on prospective observational studies and epidemiological data that link the great-est health benefits to at least three daily servings of whole grain foods. Eating more whole grain foods may be one of the healthi-est choices individuals can make to help cut their risk of prevent-able premature degenerative diseases (Jacobs and Gallaher 2004). The complex combination of vitamins, minerals, antioxidants, fiber, and other substances found naturally in whole grains ap-pears to work together to cut both the risk of heart disease and some cancers (Anderson et al 2000; McKeown et al 2002). When a grain is pearled, some of the bran and the germ are removed, resulting in losses of fiber, B vitamins, vitamin E, trace minerals, unsaturated fat, and ≈75% of the phytonutrients (Schroeder 1971). The tannins and phenolic compounds in the bran layer of red wheat give the darker color to its flour; this darker color is com-monly associated with whole grain products (Schroeder 1971). White wheat does not contain tannins in its bran. The flour from white wheat whole grain resembles typical refined flour but it has the nutrition of whole grain. Some whole grain products are being made with naturally white wheat grain. Finer particle size whole grain wheat products are being introduced to increase the whole grain consumption and to meet the color and texture preferences of the consumers. Regardless of how the whole grain is handled, a whole grain food product must deliver approximately the same relative proportions of bran, germ, and endosperm found in the original grain (AACC International 2004). According to the Na-tional Health and Nutrition Examination Survey, only 35% of Americans age 12 and over met their total grain recommendation; 4% met the current whole grain recommendation (CDC 2005). In vitro bile-acid-binding without the use of labeled isotopes is an economical method for screening various foods and food fractions to evaluate their healthful potential before initiating time- and cost-intensive animal and human studies. Bile acids are acidic steroids synthesized in the liver from cholesterol. After conjuga-tion with glycine or taurine, they are secreted into the duodenum. Bile acids are actively reabsorbed by the terminal ileum and un-dergo an enterohepatic circulation (Hofmann 1977). The bile ac-ids are needed for the absorption of dietary fat from the GI tract. The dietary fat is metabolized to acetate. Acetate is the principal precursor of cholesterol synthesis in the body. Binding of bile acids and increasing fecal excretion has been hypothesized as a possible mechanism for lowering cholesterol by dietary fiber (Trowell 1975; Lund et al 1989; Anderson and Siesel 1990). By binding bile acids, cereal fibers prevent their reabsorption and stimulate plasma and liver cholesterol conversion to additional bile acids (Eastwood and Hamilton 1968, Balmer and Zilversmit 1974, Kritchevsky and Story 1974). Toxic metabolites in the gut and secondary bile acids increase the risk of colorectal cancer (Cos-tarelli et al 2002). Healthful, cholesterol lowering (atherosclerosis amelioration, detoxification of harmful carcinogenic metabolites) potential of grains and grain fractions could be predicted by evaluating in vitro bile-acid-binding based on positive correla-tions between in vitro and in vivo studies showing that choles-tyramine (bile-acid-binding cholesterol lowering drug) binds bile acids and cellulose does not (Suckling et al 1991; Nakamura and Matsuzawa 1994; Daggy et al 1997; Kahlon and Chow 2000). This study was conducted to determine relative healthful potential of cooked whole grain wheat cultivars (hard red winter, hard white winter, and durum) and their pearled (partially refined) grains by evaluating in vitro bile-acid-binding on an equal dry matter basis, with bile acid mixture under duodenal physiological pH 6.3.

MATERIALS AND METHODS

The whole wheat grain samples, crop of 2006, were obtained from California Wheat Commission, Woodland, CA. Whole wheat grains cultivars (Hard Red Winter, cultivar Summit, location Zanola; Hard White Winter, cultivar Blanca Grande, location Ad-ams; durum, cultivar Kronos, location Casa Grande) were tested in the present study. Pearled wheat grains were obtained using 100-g samples of whole grain wheat, tempered to 15% moisture, and milled in a pearling mill (model 17810 and sieve D, Strong-Scott Pearler, Seedburo Equipment, Chicago, IL) for 2 min to partially remove bran and germ. Pearling removed 24.2, 18.3, and

[1] Western Regional Research Center, USDA, Agricultural Research Service, 800 Buchanan Street, Albany, CA 94710. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

[2] Corresponding author. Phone: 510-559-5665. Fax: 510-559-5777. E-mail address: Talwinder.Kahlon@ars.usda.gov

19.0% of hard red winter, hard white winter, and durum dry matter, respectively.

Cooking Wheat Grain

Wheat grains were cooked in boiling water in a 4-qt nonstick Teflon coated pot. Cooking time for whole grain samples was 65 min with a grain-to-water ratio of 1:2 and cooking time for pearled grain was 50 min with a grain-to-water ratio of 1:3 (Table I). Grains were cooked to level similar to that normally done for ready-to-eat rice. Evaluating the bile-acid-binding potential of ready-to-eat cereals would be desirable to determine health promoting potential of whole vs. pearled (partially refined) wheat grain. Cooked grains were dried to constant weight at 68°C in a food dehydrator for 48 hr (model 062, Proctor and Schwartz, Horsham, PA). Dry samples were ground (Thomas-Wiley Mini Mill, Arthur Thomas, Philadelphia, PA) to pass a 0.4-mm screen. Protein content of samples was determined using a combustion nitrogen analyzer (model FP 428, Leco). Moisture content was determined by Method 935.29 (AOAC 1990). Total dietary fiber (TDF) was determined by Method 985.29 (AOAC 2000). To determine TDF, duplicate test portions of dried foods were gelatinized with heat-stable α-amylase (Termamyl, Sigma, St. Louis, MO) and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. Four volumes of ethyl alcohol were added to precipitate soluble dietary fiber. Total residue was filtered and washed with 78% ethyl alcohol, 95% ethyl alcohol, and acetone. After drying, residue was weighed. One duplicate was analyzed for protein, another was incinerated at 525°C, and ash was determined. TDF was calculated by subtracting the weight of the protein and the ash from the sample residue. Cholestyramine, a bile-acid-binding anionic resin (a drug that lowers cholesterol and binds bile acids) was the positive control treatment. Cellulose (a nonbile-acid-binding fiber) was the negative control. Both were obtained from Sigma (St. Louis, MO). All the analyses were conducted in triplicate.

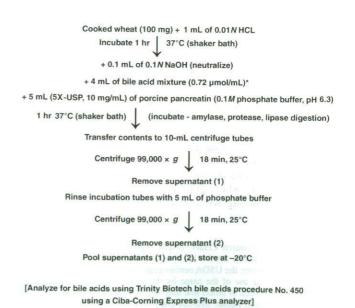## Bile-Acid-Binding Procedure

The in vitro bile-acid-binding procedure was a modification of that by Camire et al (1993) as previously reported (Kahlon and Chow 2000). The stock bile acid mixture was formulated with 75% glycocholic bile acids and 25% taurine-conjugated bile acids based on the composition of human bile (Carey and Small 1970; Rossi et al 1987). This mixture contained glycocholic acid (9 mmol/L), glycochenocholic acid (9 mmol/L), glycodeoxycholic acid (9 mmol/L), taurocholic acid (3 mmol/L), taurochenocholic acid (3 mmol/L), and taurodeoxycholic acid (3 mmol/L) in pH 6.3,

0.1M phosphate buffer. This stock solution of 36 mmol/L was stored in a –20°C freezer and diluted to the working solution (0.72 μmol/mL) just before each assay. Six replicates of 100–101 mg of dry matter of whole grain and pearled wheat samples, cholestyramine 25 mg, and cellulose 25 mg were tested. The amount of cholestyramine (25 mg) was chosen as it can bind >90% of the bile acids in the incubation mixture. The amount of dietary fiber (11–14 mg) in the test samples assures that bile acids are not limiting in the incubation mixture. One substrate blank, one positive blank (2.88 μmol of bile acid mixture/incubation without substrate) and six treatment replicates were weighed into 16 × 150 mm glass, screw-capped tubes. Samples were digested in 1 mL of 0.01N HCl for 1 hr in a 37°C shaker bath. After this acidic incubation simulating gastric digestion, the sample was adjusted to pH 6.3 with 0.1 mL of 0.1N NaOH. Each test sample received added 4 mL of bile acid mixture working solution (0.72 μmol/mL) in a 0.1M phosphate buffer, pH 6.3. A phosphate buffer (4 mL, 0.1M, pH 6.3) was added to the individual substrate blanks. After the addition of 5 mL of porcine pancreatin (5× USP specifications, 10 mg/mL, in a 0.1M phosphate buffer, pH 6.3) that provided amylase, protease, and lipase for digestion of samples, tubes were incubated for 1 hr in a 37°C shaker bath. Mixtures were transferred to 10-mL centrifuge tubes (Oak Ridge 3118-0010, Nalgene, Rochester, NY) and centrifuged at 99,000 × g in a 75-Ti rotor at 39,000 rpm for 18 min at 25°C in an ultracentrifuge (model L-60, Beckman, Palo Alto, CA). Supernatant was removed into a second set of labeled tubes. An additional 5 mL of phosphate buffer was used to rinse out the incubation tube and added to the centrifuge tube, which was vortexed and centrifuged as before. Supernatant was removed and combined with the previous supernatant tube. Aliquots of pooled supernatant were frozen at –20°C for bile acids analysis. Bile acids were analyzed using a bile acids procedure (No. 450, Trinity Biotech Distribution, St Louis, MO) using an analyzer (Ciba-Corning Express Plus, Polestar Labs, Escondido, CA). Each sample was analyzed in triplicate. Values were determined from a standard curve obtained by analyzing bile acid calibrators (Trinity Biotech No. 450-11) at 5, 25, 50, 100, and 200 μmol/L. Individual substrate blanks were subtracted and bile acid concentrations were corrected based on the mean recoveries of bile acid mixture (positive blank). A diagram of the in vitro bile-acid-binding procedure is shown in Fig. 1.

TABLE I
Cooking Time, Total Dietary Fiber (TDF), Protein, and Dry Matter in Cooked Wheat[a,b,c]

Treatment	Grain to Water	Cooking Time (min)	TDF (% db)	Protein (% db)	Dry Matter (%)
Whole Grain Wheat					
Hard Red Winter	1:2	65	13.8	14.3	96.3
Hard White Winter	1:2	65	13.8	14.2	96.5
Durum	1:2	65	12.9	15.2	95.0
Pearled Grain Wheat					
Hard Red Winter	1:3	50	11.7	13.1	97.1
Hard White Winter	1:3	50	11.0	13.1	97.0
Durum	1:3	50	11.7	13.0	96.6
Controls					
Cholestyramine	–	–	100.0	–	85.4
Cellulose	–	–	100.0	–	93.2

[a] Differences in TDF, protein, and dry matter content between wheat grain treatments were not significant.
[b] Nitrogen to protein factor used for all wheat samples was 5.7.
[c] TDF, protein, and dry matter analyses conducted in triplicate.

Cooked wheat (100 mg) + 1 mL of 0.01N HCL

Incubate 1 hr   37°C (shaker bath)

↓

+ 0.1 mL of 0.1N NaOH (neutralize)

+ 4 mL of bile acid mixture (0.72 μmol/mL)*

+ 5 mL (5X-USP, 10 mg/mL) of porcine pancreatin (0.1M phosphate buffer, pH 6.3)

1 hr 37°C (shaker bath)   ↓   (incubate - amylase, protease, lipase digestion)

Transfer contents to 10-mL centrifuge tubes

Centrifuge 99,000 × g ↓   18 min, 25°C

Remove supernatant (1)

Rinse incubation tubes with 5 mL of phosphate buffer

Centrifuge 99,000 × g ↓   18 min, 25°C

Remove supernatant (2)

Pool supernatants (1) and (2), store at –20°C

[Analyze for bile acids using Trinity Biotech bile acids procedure No. 450 using a Ciba-Corning Express Plus analyzer]

* 0.1M phosphate buffer, pH 6.3 (phosphate buffer only for blank)

Fig. 1. Diagram of bile-acid-binding procedure.

The effect of treatment was tested using Lavene's test for homogeneity and least square means were calculated. Dunnett's one-tailed test was used for comparison of cholestyramine as well as cellulose against all treatments, and differences among whole grains and pearled grains were tested for significance with Tukey's test for comparison of all possible pairs of means (SAS Institute, Cary, NC). The criterion of significance value was ($P \leq 0.05$).

RESULTS AND DISCUSSION

Total dietary fiber (TDF) content of whole grain hard red winter and hard white winter wheat was 13.8%. TDF for whole grain durum was 12.9%. TDF values for all the pearled wheat grain were 11.0–11.7%. Pearling whole wheat grain partially removed bran, endosperm, and phytonutrients and reduced TDF content by 2–3% in hard red and hard white wheat; in durum, this reduction in TDF was 1%. A small reduction in TDF content in the pearled grains could be explained by partially removing outer fiber layer.

On an equal dry matter basis (db), in vitro bile-acid-binding was significantly higher for cholestyramine and significantly lower for cellulose than all the wheat grain treatments tested (Table II). The bile-acid-binding values were similar for WHrw and WHww and significantly ($P \leq 0.05$) higher than WDU and all the pearled grain treatments. Binding values were similar for WDU or PDU and PHrw and PHww. Assigning a bile-acid-binding value of 100%

TABLE II
In Vitro Bile-Acid-Binding of Cooked Whole Grain
vs. Pearled Wheat (db)[a,b]

Treatment	Bile-Acid-Binding (µmol/100 mg, db)	Bile-Acid-Binding Relative to Cholestyramine (%)
Whole grain wheat		
Hard Red Winter	0.80 ± 0.01b	7.72 ± 0.11b
Hard White Winter	0.77 ± 0.01b	7.50 ± 0.06b
Durum	0.57 ± 0.01c	5.50 ± 0.09c
Pearled grain wheat		
Hard Red Winter	0.62 ± 0.01c	6.01 ± 0.05c
Hard White Winter	0.65 ± 0.01c	6.32 ± 0.08c
Durum	0.56 ± 0.01c	5.44 ± 0.07c
Controls		
Cholestryramine	10.32 ± 0.07a	100.00 ± 0.66a
Cellulose	−1.15 ± 0.06d	−11.14 ± 0.56d

[a] Mean ± SEM (standard error of measurements) values within a column followed by different letters are significantly different ($P < 0.05$) ($n = 6$).
[b] Dry matter used for incubation for all grain samples (100–101 mg); cholestyramine (25 mg); and cellulose (25 mg).

TABLE III
In Vitro Bile-Acid-Binding of Cooked Whole Grain vs. Pearled Wheat
Total Dietary Fiber (TDF) Basis (db)[a,b]

Treatment	Bile-Acid-Binding (µmol/100 mg, TDF)	Bile-Acid-Binding Relative to Cholestyramine (%)
Whole grain wheat		
Hard Red Winter	5.77 ± 0.08b	55.93 ± 0.78b
Hard White Winter	5.62 ± 0.07c	54.43 ± 0.42c
Durum	4.38 ± 0.07f	42.46 ± 0.70f
Pearled grain wheat		
Hard Red Winter	5.29 ± 0.04d	51.24 ± 0.43d
Hard White Winter	5.91 ± 0.07b	57.25 ± 0.69b
Durum	4.79 ± 0.06e	46.44 ± 0.59e
Controls		
Cholestryramine	10.32 ± 0.07a	100.00 ± 0.66a
Cellulose	−1.15 ± 0.06g	−11.14 ± 0.56g

[a] Mean ± SEM (standard error of measurements) values within a column followed by different letters are significantly different ($P \leq 0.05$) ($n = 6$).
[b] TDF used for incubation for whole grain samples (13–14 mg), refined grains (11–12 mg).

to cholestyramine, the relative bile-acid-binding (db) for cooked wheat grains tested was WHrw, WHww, WDU, PHrw, PHww, and PDU 8, 8, 6, 6, 6, and 5%, respectively. On a dry matter basis, relative 8% bile-acid-binding by WHrw and WHww is very encouraging. Similar bile-acid-binding of hard white wheat to that of hard red suggest that the red color commonly associated with whole grain wheat may not necessarily indicate more healthfulness. Beta et al (2005) reported that bran layer of the red and white cultivars of wheat had similar phenolic content. Significantly lower bile-acid-binding by WDU than WHrw or WHww may suggest that these whole gain bread cultivars of wheat are more healthful than durum. WDU bile-acid-binding values were only slightly higher than PDU. Significantly higher bile-acid-binding values of whole grain wheat (HRw, Hww) compared with pearled grains, support epidemiological observations that whole grains are more healthful. Relative bile-acid-binding of 6–9% for oat bran, oat bran ready-to-eat cereal, and barley fractions (cereals with U.S. FDA-approved food label health claim for lowering cholesterol) have been reported (Kahlon 2003a,b). Data support the validity of the FDA-approved food label health claims for whole grains.

The bile-acid-binding on equal total dietary fiber (TDF) basis is shown in Table III. Cholestyramine bound bile acids significantly higher and cellulose bound bile acids significantly lower than various cooked whole grain and pearled grain wheat tested. On a a TDF basis considering cholestrymine as 100% bound, the bile-acid-binding values were 57% PHww, 56% WHrw, 54% WHww, 51% PHww, 46% PDU, and 42% WDU. Bile-acid-binding on a TDF basis among various wheat grains tested were PHww = WHrw > WHww > PHrw > PDU > WDU. Except for PHww and WHrw, where values were similar, there were significant differences in the bile-acid-binding values of the cooked wheat grains tested. This data is in agreement with previous observations than bile-acid-binding is not related to the TDF content (Kahlon et al 2003a,b; 2006a,b). Differences in bile-acid-binding may relate to unique combination of micronutrients, phenolic content, and phytonutrients present in bran layer of hard red and hard white wheat.

CONCLUSIONS

Whole grain wheat Hrw and Hww have significantly higher health-promoting potential than refined grains. Similar bile-acid-binding of WHww and WHrw suggest that WHww wheat was as healthful as WHrw. Darker color of hard red wheat flour commonly associated with whole grain may not necessarily indicate more healthfulness than whole grain white wheat flour. Significantly higher bile-acid-binding of WHrw and WHww suggest that regular consumption of whole grain wheat should be encouraged. It could lower the risk of cardiovascular disease and cancer, advance human nutrition research, and improve public health.

LITERATURE CITED

AACC International. 2004. AACC to Create Consumer-Friendly Definition of Whole Grains. Press Release, March 5, 2004. Available at: http://www.aaccnet.org/news/CFWholeGrain.asp.
Anderson, J. W., and Siesel, A. E. 1990. Hypocholesterolemic effects of oat products. Pages 17-36 in: New Developments in Dietary Fiber: Physiological, Physiochemical, and Analytical Aspects. I. Furda and C. J. Brine, eds. Plenum Press: New York.
Anderson, J. W., Hanna, T. J., Peng, X., and Kryscio, R. J. 2000. Whole grain foods and heart disease risk. J. Am. Coll. Nutr. 19:291S-299S.
AOAC. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th Ed. Pages 70, 725, 1st Suppl. P 83. The As-

sociation: Arlington, VA.

AOAC. 2000. Official Methods of Analysis of the Association of Official Analytical Chemists. 17th Ed. Pages 78E-80E [Chap. 45]. The Association: Arlington, VA.

Balmer, J., and Zilversmit, D. B. 1974. Effect of dietary roughage on cholesterol absorption, cholesterol turnover and steroid excretion in the rat. J. Nutr. 104:1319-1328.

Beta, T., Nam, S., Dexter, J. E., and Sapirstein, H. D. 2005. Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. Cereal Chem. 82:390-393.

Camire, M. E., Zhao, J., and Violette, D. A. 1993. In vitro binding of bile acids by extruded potato peels. J. Agric. Food Chem. 41:2391-2394.

Carey, M. C., and Small, D. M. 1970. The characteristics of mixed micellar solutions with particular reference to bile. Am. J. Med. 49:590-608.

CDC. 2005. National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Data. U.S. Department of Health and Human Services. Centers for Disease Control and Prevention: Hyattsville, MD.

Costarelli, V., Key, T. J., Appleby, P. N., Allen, D. S., Fentiman, I. S., and Sanders, T. A. 2002. A prospective study of serum bile acid concentrations and colorectal cancer risk in post-menopausal women on the island of Guernsey. Br. J. Cancer 86:1741-1744.

Daggy, B. P., O'Connell, N. C., Jerdack, G. R., Stinson, B. A., and Setchell, K. D. 1997. Additive hypocholesterolemic effect of psyllium and cholestyramine in the hamster: Influence on fecal sterol and bile acid profiles. J. Lipid Res. 38:491-502.

Eastwood, M. A., and Hamilton, D. 1968. Studies on the adsorption of bile acids to non-absorbed components of diet. Biochim. Biophys. Acta 152:165-173.

Hofmann, A. F. 1977. The enterohepatic circulation of bile acids in man. Clin. Gastroenterol. 6:3-24.

Jacobs, D. R., and Gallaher, D. D. 2004. Whole grain intake and cardiovascular disease: a review. Curr. Atheroscler. Rep. 6:415-425.

Kahlon, T. S., and Chow, F. I. 2000. In vitro binding of bile acids by rice bran, oat bran, wheat bran and corn bran. Cereal Chem. 77:518-521.

Kahlon, T. S., and Woodruff, C. L. 2003a. In vitro binding of bile acids by rice bran, oat bran, barley and β-glucan enriched barley. Cereal Chem 80:260-263.

Kahlon, T. S., and Woodruff, C. L. 2003b. In vitro binding of bile acids by various ready to eat breakfast cereals. Cereal Foods World 48:73-75.

Kahlon, T. S., Berrios, J. J., Smith, J. E., and Pan, J. L. 2006a. In vitro bile acid binding capacity of wheat bran extruded at five specific mechanical energy levels. Cereal Chem. 83:157-160.

Kahlon, T. S., Berrios, J. J., Smith, J. E., and Pan, J. L. 2006b. In vitro bile acid binding capacity of milled wheat bran and milled extruded wheat bran at five specific mechanical energy levels. Cereal Chem. 83:434-438.

Kritchevsky, D., and Story, J. A. 1974. Binding of bile salts in vitro by nonnutritive fiber. J. Nutr. 104:458-462.

Lund, E. K., Gee, J. M., Brown, J. C., Wood, P. J., and Johnson, I. T. 1989. Effect of oat gum on the physical properties of the gastrointestinal contents and on the uptake of D-galactose and cholesterol by rat small intestine in vitro. Br. J. Nutr. 62:91-101.

McKeown, N. M., Meigs, J. B., Liu, S., Wilson, P., and Jacques, P. F. 2002. Whole-grain intake is favorably associated with metabolic risk factors for type 2 diabetes and cardiovascular disease in the Framingham Offspring Study. Am. J. Clin. Nutr. 76:390-398.

Nakamura, T., and Matsuzawa, Y. 1994. Drug treatment of hyperlipoproteinemia: Bile acid-binding resins. Nippon Rinsho 52:3266-3270.

NHANES. 2005. Dietary Guidelines for Americans, 6th Ed. USDA: Washington DC.

Rossi, S. S., Converse, J. L., and Hoffman, A. F. 1987. High pressure liquid chromatography analysis of conjugated bile acids in human bile: Simultaneous resolution of sulfated and unsulfated lithocholyl amidates and the common conjugated bile acids. J. Lipid Res. 28:589-595.

Schroeder, H. A. 1971. Losses of vitamins and trace minerals resulting from processing and preservation of foods. Am. J. Clin. Nutr. 24:562-573.

Suckling, K. E., Benson, G. M., Bond, B., Gee, A., Glen, A., Haynes, C., and Jackson, B. 1991. Cholesterol lowering and bile acid excretion in the hamster with cholestyramine treatment. Atherosclerosis 89:183-190.

Trowell, H. C. 1975. Refined Foods and Disease. Pages 195-226. D. P. Burkitt and H. C. Trowell, eds. Academic Press: London.